Second. The cases occurred in widely separated parts of the city, and in no instance was there the slightest suspicion of association.

Third. After the cases were brought to the attention of the Health Department, in no instance did a secondary case occur.

Fourth. There was a striking association in the occupation of the persons affected with the food trades, and especially animal foods.

Fifth. Although some twenty-six suspicious cases were examined, in every one in which the cholera spirillum was found, eight in number, death occurred, while in only one of the other cases, in which the cholera spirillum was not found, did death follow. In two instances, however, where recovery took place—that is, the fifth and sixth cases—although biological examinations were not made, the associations with cases which were proven to be cholera Asiatica justified the assumption that these were of the same nature.

THE BACTERIOLOGICAL EXAMINATION OF THE RECENT CASES OF EPIDEMIC CHOLERA IN THE CITY OF NEW YORK.

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The materials submitted for bacteriological examination differed considerably in character. As most of the suspected cases died after a very short illness, material could first be collected only at the autopsy. Some of the intestinal contents, and in one case some of the contents of the stomach, were collected in vessels for transmission to the laboratory, or the ileum was tied at two points a short distance apart and then cut beyond the ligatures so that there might be the least possible alteration of the contents before its actual examination. In two cases fluid dejecta were obtained from suspected cases during life and sent to the laboratory in stoppered bottles. In still other cases the only available material before the death of the individual was clothing soiled by dejecta.

The method of examination necessarily differed according to the nature of the material received for examination. Where this material was fluid it was examined directly under the microscope, without previous staining, and again after drying and dyeing with aniline colors.

These direct microscopical examinations of the intestinal contents proved very unsatisfactory. In only one case did cover-glass preparations of the dejecta show large numbers of comma bacilli agreeing in form and size with the cholera bacillus. This case was where dejecta had been collected from a patient some time before death and examined within a very few hours. Plate cultures from the same material showed that the cholera bacillus made up 98 per cent. of those bacteria present which were capable of developing in ordinary nutrient gelatin at the temperature of the room.

In all the other cases microscopical examination revealed an abundance of columnar epithelium from the intestinal mucous membrane; numerous straight, thick bacilli; a considerable amount of granular detritus; but at most only a few curved bacilli, which could not warrant even a presumption for or against the probability of a diagnosis of epidemic cholera. Plate cultures from this same material revealed the presence of the cholera bacillus in proportions varying from about 20 per cent. to as high as 90 per cent. of the total bacteria developing upon the plates. This observation shows the importance of not relying upon a simple microscopical examination of dejecta or intestinal contents. An analogy is presented by the untypical forms found in old bouillon and gelatin cultures of the cholera bacillus.

The cultures made for the purpose of identifying the cholera bacillus were invariably prepared by inoculation from typical colonies upon plate cultures. These plate cultures were made, where possible, directly from the fresh dejecta, or from material taken from the intestine or stomach at the autopsy. Where this was impossible, the material sent for examination (soiled clothing, etc.) was introduced into bouillon in test-tubes, and the latter placed in an incubator at 36° C. (96.8° F.) for six, eight, or twelve hours, until the bouillon was cloudy. From these impure bouillon cultures the initial plate cultures were then made, and the colonies developing on these plates used to inoculate other media for the purpose of identification.

In order not to miss the cholera bacillus, should it be present in small proportion, even where dejecta or intestinal contents were received, this preliminary bouillon cultivation was made in the hope that in this way material for plate cultures, more favorable than the original material, might be obtained. This safeguard against possible failure to detect the cholera bacillus in the original material proved unnecessary in all the cases examined. The first set of plates from the original dejecta always yielded positive results, there being no difficulty in detecting the colonies of cholera bacilli. A previous cultivation in bouillon at the body temperature appeared, however, to increase somewhat the relative abundance of the cholera colonies upon the plates. In the cases where the material to be examined was very small in amount and dried upon clothing, a preliminary cultivation in bouillon proved very satisfactory.

The bacteria associated with the cholera bacillus were chiefly bacterium coli commune or proteus vulgaris, so that there was no difficulty in distinguishing those presumably cholera from those which were certainly not cholera. But the appearances of the cholera colony varied so much on plates which developed at different temperatures, and with the size of the diaphragm used when they were examined under the microscope, that it seems excusable to dwell a little upon these variations, since the recognition of the cholera colony is the first step in the bacteriological diagnosis of a case.

The classical descriptions of the cholera colony refer to colonies which have developed at a temperature of from 60° to 65° F. Under these conditions the colony appears circular, coarsely granular, with a peculiar high refraction, giving an appearance as though the colony were made up of broken bits of glass or beads of various sizes grouped together. It is colorless, or sometimes has a very faint pinkish tinge when examined with some lenses, but not, as far as I have observed, when examined with apochromatic objectives. Around the colony, after it has reached a considerable size (thirty-six to forty-eight hours), there is a narrow zone, in which the gelatin is softened and its surface depressed. This causes a refraction of the light, so that the colony appears surrounded by a shadow when a moderately small diaphragm is used.[1]

This description of the colony applies only to colonies more than twenty-four hours old. Very young colonies appear as minute, fine granular, colorless spots; nearly circular, but with a slightly irregular edge.

Colonies which have developed at a higher temperature, approaching the point at which the gelatin begins to soften (from 70° to 73° F.), show considerable modifications attributable to the more rapid liquefaction of the gelatin. Instead of forming a compact mass surrounded by only a narrow zone of softening, the growth appears more or less scattered through a circular area of liquefaction. There is a central, more compact mass, resembling the colony grown at a lower temperature; but its edge is ragged, and in the liquid surrounding it there are small, irregular portions of the growth which appear to have been detached from the central mass, and to float about freely in the liquid. The edge of the liquefaction, when a very small diaphragm is used, shows a narrow border of growth, with very fine short radiations resembling the milled edge of a coin, but never extending appreciably into the solid gelatin.

The appearances described are illustrated in Figs. 1 to 4.

As soon as the colonies upon the plate cultures had grown enough to develop their characteristic appearances, material from them was used to inoculate tubes containing about two cubic centimetres of a sterile solution of 1 per cent. peptone and ½ per cent. table salt in distilled water. These peptone cultures were then placed in the incubator at about 36° C. (96.8° F.) for six hours. They were then examined in hanging-drop preparations to determine the form, arrangement, and motility of the individual bacteria. In all of the cases these hanging drops showed curved bacilli, either isolated or in S-shaped pairs, and possessed of a very active motility. The growth was sufficiently abundant to render the liquid distinctly cloudy, and to occasion a whirling appearance in the cloudiness when the tube was shaken. These tubes were then used to obtain the "indol reaction" by pouring a few drops of concentrated, "strictly pure" sulphuric acid down the inner surface of the tube, so that a layer of the strong acid was formed below the cultures. Within a few moments the peptone solution acquired a distinct rose tint, sometimes tending toward lilac. In such young cultures the color was often faint, but in all cases it was unmistakable. The peptone used was Witte's "peptonum siccum."

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[1] The Abbé condenser with iris diaphragm was used when the plates were examined, and the size of the diaphragm varied to bring out the structure of the colonies. The smallest aperture was about one millimetre.

These observations required from thirty-six to forty-eight hours from the time the material was received, and if all the data furnished by them agreed with the known characteristics of the cholera bacillus, a positive report was considered as justified. These data were: 1. The size, form, and arrangement of the individual bacilli. 2. Their motility. 3. The appearance of colonies upon plate cultures. 4. The comparatively slow liquefaction of 10 per cent. slightly alkaline nutrient gelatin. 5. The rapid development in peptone solution at the body temperature. 6. An early and distinct development of a positive response to the "indol test."

Fortunately, in all the cases examined, the variations in the above characteristics fell within the limits of variation previously well recognized in studies of fresh, pure cultures of the cholera bacillus grown at various temperatures. There was, therefore, no ground for hesitation in arriving at a diagnosis.[1]

As confirmatory tests, cultures in gelatin tubes, on agar-agar, and on potato were also made.

The gelatin-tube cultures show variations in development perfectly analogous to those observed in the colonies upon plate cultures, and due also to differences of temperature. These variations are of degree rather than of kind, and are illustrated in the accompanying photographs.

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[1] In further confirmation of my identification of the cholera bacillus in cultures from the recent cases in New York, I have received communications from Dr. Harold C. Ernst, of Harvard University Medical School, and from Dr. Petri, of the Imperial Board of Health in Berlin, stating that the cultures which I had the pleasure of sending them from the first case (McAvoy), and which they very kindly studied, were, in their opinion, characteristic of cholera. Dr. Petri further stated that after comparing my culture with recent cultures from cases in Hamburg and in Berlin for two weeks, he could detect *no difference* between them. I wish here to express my thanks to these gentlemen for their kindness in this matter.

Not only do the appearances of cultures differ where the development has taken place under different conditions, but the appearances of the individual bacilli vary somewhat according to the medium and the temperature. Under some circumstances the bacilli appear short and but slightly curved. In other cases they are longer, appear more slender, and show a much more marked curvature. These observations are illustrated by the photographs. Fig. 10 was taken of a contact cover-glass preparation made from a plate culture after development for forty hours at a rather high temperature (between 65° and 70° F.). Fig. 11 is a photograph of a cover-glass preparation made from a culture in peptone solution. Fig. 12 is a photograph from a cover-glass preparation made by contact from a plate culture grown at a low temperature. It is very exceptional to find spirilla in colonies developed upon gelatin plates. In bouillon cultures of cholera from the first case (McAvoy), spirilla of twenty-five turns were observed.

The following is a brief summary of the results obtained in the individual cases which yielded positive results:

Sample 1 [Case I.; McAvoy.]—Intestinal contents obtained from the ileum more than twenty-four hours after death. Cholera bacilli found in the proportion of about 30 per cent. of all the bacteria growing upon plate cultures.

Sample 2 [Case IV.].—Dejecta. Cholera bacilli found in great abundance. Percentage not determined.

Sample 3 [Case III.].—Dejecta. Examination within a few hours of collection. 98 per cent. cholera bacilli.

Sample 4 [Case VII.].—Intestinal contents received in a portion of colon tied at both ends, more than twenty-four hours after death. Cholera bacilli abundant. No record of percentage.

Sample 5 [Case IX.].—Two samples: 1. Rag soiled with dejecta. 2. Cotton wad soaked in a mixture of dejecta and vomitus which had been very largely diluted with water by the family of the patient. Cholera found in abundance in both samples. Percentage estimation impossible. Large quantities of proteus vulgaris contaminated the plate cultures.

Sample 6 [Case VIII.].—Intestinal contents. Cholera bacilli in great abundance. No record of percentage.

Sample 7 [Case X.].—Dejecta on clothing and on wad of absorbent cotton. Cholera bacilli in great abundance. Percentage determination impossible.

Sample 8 [Case XI.].—Dejecta examined four hours after their collection. 32 per cent. cholera. Intestinal contents examined within twelve hours after death. 88 per cent. cholera.

Sample 9.—Intestinal contents examined about twenty-four hours after death. 85 per cent. cholera. The gastric contents contained 70 per cent. of cholera.

In every case the examination was made in duplicate; in most of the cases in triplicate or quadruplicate.

FIG 1.

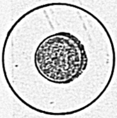


FIG. 2.

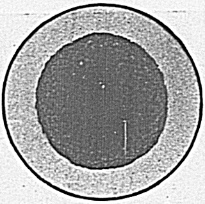


FIG. 3.

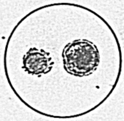


FIG. 4.

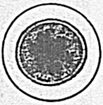


FIG. 5.

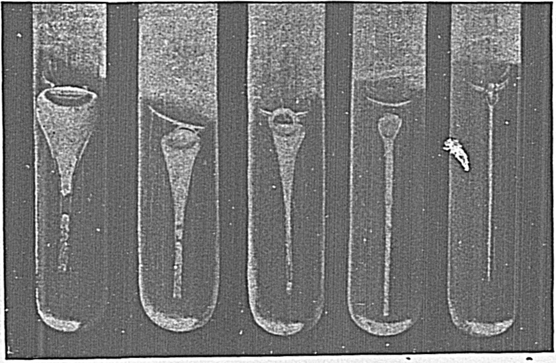


FIG. 6.

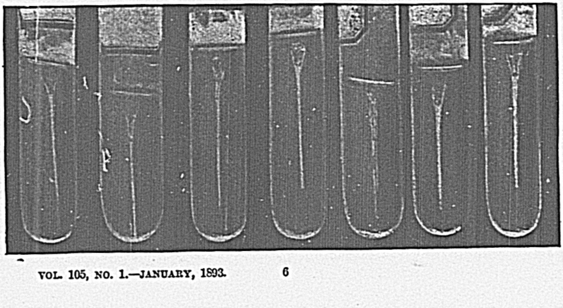


Fig. 7.

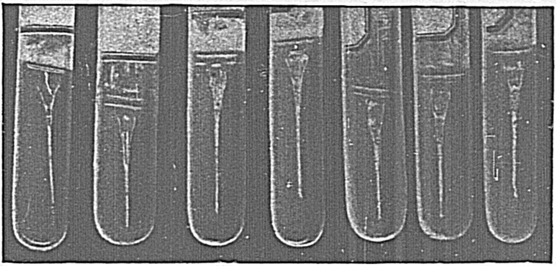


Fig. 8.

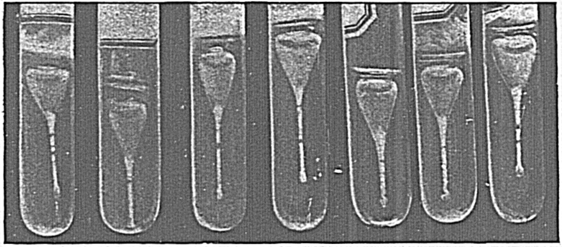


Fig. 9.

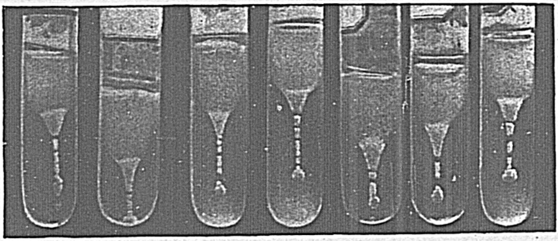


FIG. 10.

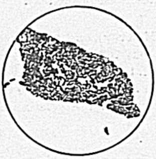


FIG. 11.

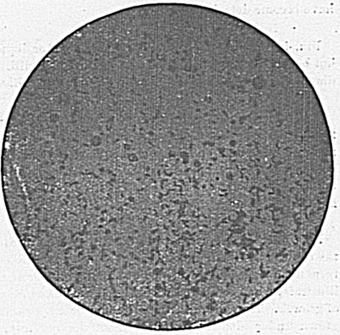
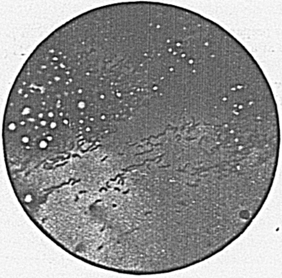


FIG. 12.



EXPLANATION OF THE ILLUSTRATIONS.

FIG. 1. Colony of cholera bacilli on a plate culture forty hours after its preparation. Photographed with Abbé condenser and small diaphragm. The refraction of the light by the depressed surface of the softened gelatin around the colony is perceptible. Amplification about 40 diameters. Culture obtained from intestinal contents of the first case.

FIG. 2. Colony of cholera bacilli on a plate culture two days old. Magnified about 75 diameters. Small diaphragm.

FIG. 3. Two colonies of cholera bacilli on a plate culture forty hours old. One of the colonies shows an extension of the liquefaction for a short distance beyond the central mass of growth. The edge of the central mass is ragged, and portions of the growth have become detached.

FIG. 4. Cholera colony on a plate culture after liquefaction of the gelatin had begun, but had not extended beyond the colony of bacilli. Photographed with a very small diaphragm. The edge of the colony shows the milled effect described in the text.

FIG. 5. Gelatin-tube cultures of cholera from the first case (McAvoy), one, two, three, four, and six days old. Temperature during development about 70° F. The culture medium was prepared from a cold aqueous extract of lean beef, to which 1 per cent of peptone, ½ per cent. of salt, and 10 per cent. of gelatin had been added, according to the methods usual in the Hygienic Institute in Berlin. The reaction of the gelatin was faintly alkaline.

FIGS. 6, 7, 8, and 9 are parallel gelatin-tube cultures from the first seven cases in which the cholera bacillus was found. They were inoculated at the same time from pure cultures upon agar, and developed at a temperature ranging between 50° and 60° F. They illustrate, when compared with Fig. 5, the influence of temperature upon the rapidity of growth. They also illustrate the identical appearance of cultures of the same bacillus grown under identical conditions, but derived from different sources. These photographs were taken after three, five, nine, and fifteen days' development.

FIG. 10. Contact cover-glass preparation from a plate culture of cholera after forty hours' development. Magnified about 700 diameters.

FIG. 11. Cover-glass preparation from a culture of cholera in peptone solution, developed for twelve hours at a temperature of 36° C. (96.8° F.). Magnified 700 diameters.

FIG. 12. Contact cover-glass preparation from a plate culture developed at a low temperature. Amplification about 700 diameters.